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Armillaria in the Northern Rockies: Delineation of Isolates Into Clones

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RESEARCH SUMMARY

A method for segregating a large population of *Armillaria* spp. isolates into clones is described. The method includes statistical analysis as a part of clonal definition. Four kinds of errors were investigated: readers could either (1) agree to or (2) disagree with theory, or disagree when (3) compatibility was expected or (4) when incompatibility was expected. All four errors occurred about 4 percent of the time. The error rate was high enough to require statistical tests of delineation efficiency.

A population of 394 isolates was reduced to 177 clones. Traits common to the clones allowed further condensation into four groups. Ecological behavior (host affinity - rhizomorph production) of these groups suggests that the Northern Rockies may support four *Armillaria* spp. In addition, certain cases of isolates showing compatibility over extended geographic distances suggest the population may contain haploid isolates. The large number of multiple-membered clones is a valuable research resource that will advance the technology of species identification within the genus *Armillaria*.

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INTRODUCTION

Soil-inhabiting fungi belonging to the genus *Armillaria* cause root disease and decay on most woody plant species throughout the world (Wargo and Shaw 1985). Recent findings have contributed to an understanding of the taxonomy (Korhonen 1978; Morrison and others 1985; Ullrich and Anderson 1978), physiologic interaction (Wargo 1984), and ecological relationships (McDonald and others 1987) of the genus.

Knowledge about distribution and behavior of *Armillaria* spp. in several regions has appeared in recent years. Two reports published in 1978 outlined the concept of intersterility groups and mapping of the geographic extent of clones. One dealt with the genus in the northeastern portion of North America (Ullrich and Anderson 1978) and the other with *Armillaria* in Finland (Korhonen 1978). The above authors outlined necessary technology to map the extent of both vegetative and sexually active clones. Isolates will fail to join together because they belong to different intersterility groups or they are different genotypes within the same group.

These reports, together with more recent information, supply technology necessary to tentatively identify the taxonomic affiliation of vegetatively delineated clones, provided that fresh basidiospores of all species represented in the vegetative population are available. Six intersterile groups have been identified in Europe. Their taxonomic affinities were demonstrated using these techniques. The groups are as follows: Korhonen's (1978) group D = *Armillaria mellea* (Vahl:Fr.) Kummer, group A = *A. borealis* Marxmuller et Korhonen, group E = *A. bulbosa* (Barla) Watling, group B = *A. cepistipes* Velenosvsky, and group C = *A. ostoyae* (Romagnesi) Herink (Roll-Hansen 1985).

Ten intersterility groups were found in North America (Anderson and Ullrich 1979). Two of the 10 were later consolidated with two others, which resulted in eight groups (Anderson 1986). In the meantime, an additional intersterile group was proposed (Morrison and others 1985). Genetic and taxonomic connections were recently reported among *A. mellea* (North American group VII) growing in the State of Virginia and Korhonen's group D. In addition these connections were shown between *A. bulbosa* (North American group VII) growing in the State of Maryland and Korhonen's group E (Motta and Korhonen 1986). A definite connection was made between Korhonen's group C (*A. ostoyae*) and North American group I (Anderson and others 1980; Morrison and others 1985). Finally, a tentative connection was shown in Anderson and others' (1980) data between *A. bulbosa* and North American group VII and between *A. cepistipes* and North American group X.

These gains in clarifying the identity of *Armillaria* isolates are significant, but several points of confusion still exist. Most workers (Adams 1974; Kile 1981; Korhonen 1978; Shaw and Roth 1976) have limited their studies to rhizomorphic and fan collections from diseased plants or stumps in areas of obvious infection or, if collections covered a wider geographic area, they were limited to fruiting bodies (Anderson and Ullrich 1979; Morrison and others 1985). These sampling philosophies may provide a biased view of *Armillaria* because all populations do not fruit regularly and because potentially pathogenic populations can exist in a nonfruiting status on apparently healthy hosts. Korhonen (1978) concluded that clonal delineation of isolates in Finland by both vegetative and genetic means gave nearly identical results. In terms of clone size, both methods gave similar results in Western North America (Anderson and others 1979).

Effective management of forests where *Armillaria* is a potential threat requires the answers to several basic questions. Is risk uniform over a forested area or does it vary, and can it be predicted by applying certain criteria? Are all hosts at equal risk? If not why not? Do certain forest management actions increase the problem and others reduce it? Answers to these questions require knowledge about the distribution and behavior of the genotypes, ecotypes, and species of the causal organism over a large area. A study was conceived to answer some of these questions as they pertain to an area 600 miles in diameter, centered on Moscow, ID. Because our goal was to characterize the *Armillaria* population over the entire area, initial sampling was limited to vegetative structures. This paper reports the delineation of clones and their geographic and host range from *Armillaria* isolates sampled as vegetative structures on eighty-seven 0.04-hectare random plots located on 12 National Forests.

MATERIALS AND METHODS

Plot selection, plot location, and fungus isolation procedures were described previously (McDonald and others 1987). Contaminant-free isolates (individual pure cultures) of the genus were tested for compatibility without knowing the identity of the isolates (blind challenge test) in the following fashion: Up to six isolates were inoculated onto 3 percent malt extract agar (2 percent agar) in 50-mm plastic culture (petri) plates according to the pattern shown in figure 1. A 1-mm plug of each isolate was cut from the edge of an actively growing colony and placed 5 mm from its neighbor. All field isolates were presumed to

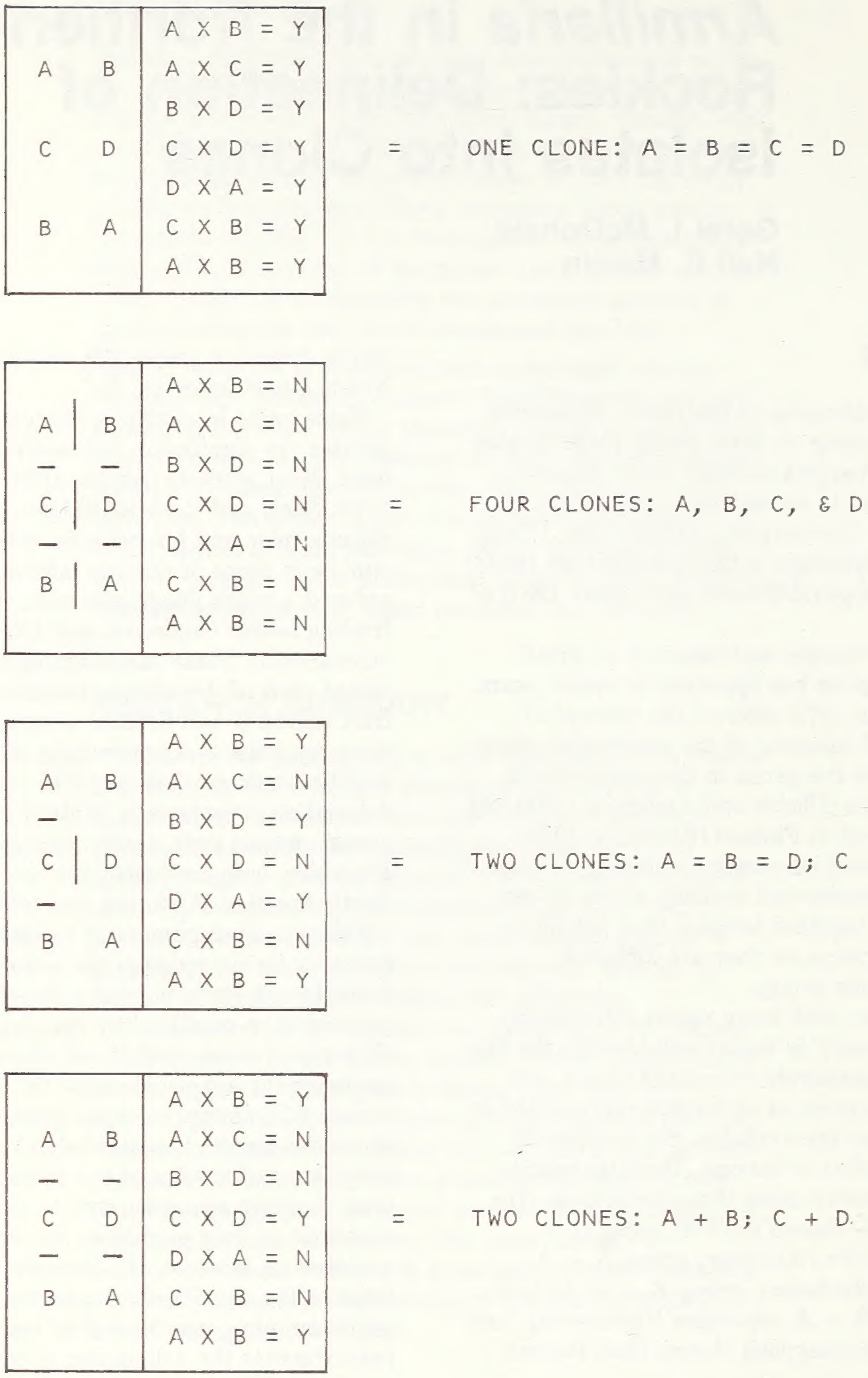

Figure 1—Sample illustrations of pattern of isolate challenges used to delineate clones of the genus *Armillaria* found on National Forests of the Northern Rocky Mountains.

be stable diploids (Korhonen 1978). The plates were incubated under room conditions for 21 to 30 days prior to reading and recording results. Each challenge was replicated three times to produce a single evaluation (read). Each of us independently assessed the three replications to record a summation of "yes" (compatible) or "no" (noncompatible). The three replications were judged "yes" if the challenge mates grew freely together and "no" if they failed to grow together, as shown by a line of demarcation, a free space, or a line resulting from mycelium crowding. A crowd line is formed when the challengers push against each other but appear not to join and they do not form a brown line of demarcation. Genetically identical mycelia are thought to freely grow together (Korhonen 1978). Relationships between isolates that failed to grow together could range from parents and sibs with identical compatibility alleles but otherwise different genomes to species that belong to different intersterility groups (Korhonen 1978).

PREMISE FOR CLONE DEFINITION

SAMPLE CHALLENGES OF 10 CULTURES FROM PLOT FH-4

1ST PREMISE CHALLENGE:	175 X	176	177	178	180	181	182	183	184	185	186
RESULT:		N	N	N	Y	Y	Y	N	Y	Y	N
REORDER:		180	181	182	184	185	176	177	178	183	186
RESULT:		Y	Y	Y	Y	Y	N	N	N	N	N

2ND PREMISE CHALLENGE:	176 X	177	178	183	186
RESULT:		N	Y	Y	N
REORDER:		178	183	177	186
RESULT:		Y	Y	N	N

3RD PREMISE CHALLENGE:	177 X	186
RESULT:		N

CONCLUSION: 4 CLONES PROVISIONALLY DEFINED

FH-41 = 175, 180, 181, 182, 184, 185
FH-42 = 176, 178, 183
FH-43 = 177
FH-44 = 186

Figure 2—Detailed premise analysis of challenge results obtained from vegetative isolates of the genus *Armillaria* found on 0.04-ha plot No. 4 located on the Flathead National Forest in Montana (FH-4).

The blind read was accomplished by working in three-person teams. One constructed the combinations of challenges and labeled (sequentially) each set of six isolates with a challenge number. The isolate numbers and associated challenge number were recorded, but their plot identification and other data were kept separated. The first round of challenges was accomplished over a period of 12 months, with individual challenges being repeated up to 10 times. These challenges were made in random combinations among the isolates from within a National Forest. Next, Punnett squares were constructed for all the isolates within each plot from within each National Forest. Blind challenges were then constructed to complete preliminary clonal definitions (premise-challenge) of the isolate populations from each plot (figs. 2, 3). Further blind challenges were made to complete the verification regions of each Punnett square (fig. 3). The evaluations by the two of us were recorded separately. Thus, each cell of the square could have a mixture of yes and no responses.

SAMPLE OF CLONAL-DEFINITION PUNNETT SQUARE: PLOT FH-4

CLONES	FH-41						FH-42			FH-43	FH-44
ISOLATES	175	180	181	182	184	185	176	178	183	177	186
PREMISE	*175*	*4Y*	*4Y*	*4Y*	*2Y*	*2Y*	*18N*	*3N1Y*	*12N*	*7N1Y*	*4N*
VERIFY		180	16Y	2Y	2Y	6Y	10N	6N2Y	3N1Y	4N	2N
			181	2Y	2Y	6Y	4N	4N	1N1Y	4N	6N
				182	2Y	2Y	4N	4N	13N1Y	4N	2N
					184	10Y	2N	2N	2N	2N	2N
						185	2N	2N	2N	4N	6N
PREMISE							*176*	*6Y*	*4Y*	*8N*	*2N*
VERIFY								178	22Y2N	26N2Y	2N
									183	15N1Y	2N
PREMISE										*177*	*4N*

Figure 3—Detailed premise and verification analysis of vegetative isolates of the genus *Armillaria* found on plot FH-4.

Given the total number of determinations in a plot and the pattern that developed, the observed number of yes and no responses was tested against the expected number (fig. 4). If the fit was statistically satisfactory, the provisional clonal delineation was accepted.

Behavior of each clone was assessed by its pattern of occurrence as saprophytic fans or epiphytic rhizomorphs on dead conifers, as parasitic fans or epiphytic rhizomorphs on *Armillaria*-infected or killed conifers, and as epiphytic rhizomorphs on apparently healthy hardwoods and conifers. Chi-square contingency tables with correction for continuity (Snedecor 1956) were used to test these relationships. Geographic range of individual clones within a National Forest was investigated by challenging randomly selected isolates within clones across plots within a National Forest.

RESULTS

Clone Definitions

Because it is impossible to present the complete analysis of 70 plots, we will discuss one plot in detail and summarize the rest. Plot 4 from the Flathead National Forest was selected because it included several clones, two of which were represented by multiple isolates. The premise-challenges (figs. 2, 3, and 4) clearly defined four clones. The verification-challenges clearly supported the definitions (plot FH-4 in table 10, appendix). Clone FH-41 (Flathead N.F.-plot 4, clone number 1) was present exclusively on *Armillaria*-killed or fading Douglas-fir (*Pseudotsuga menziesii*), Englemann spruce (*Picea engelmannii*), and subalpine fir (*Abies lasiocarpa*) as both mycelial fans and epiphytic rhizomorphs. Clone FH-42 was present as epiphytic rhizomorphs on healthy western larch (*Larix occidentalis*), Douglas-fir, and maple (*Acer* sp.). Clone FH-43 was present as epiphytic rhizomorphs on healthy willow (*Salix* sp.). Clone FH-44 was growing epiphytically on a healthy subalpine fir.

In total, 377 isolates from 70 plots were delineated into 160 clones. An additional 17 single-isolate plots were added to make the total 394 isolates, 177 clones, for 87 plots. Of the 70 plots supplying two or more isolates, two that were supporting five clones failed to yield acceptable clonal definitions (plots FH-6 in table 4 [see section The Challenges] and LO-2A in table 13, appendix). Thus, five of the 177 clones were not adequately defined and were removed from the data for the remaining analyses.

CHI-SQUARE GOODNESS-OF-FIT TEST FOR CLONAL DELINEATION OF ISOLATES FROM PLOT: FH-4

PROVISIONAL DEFINITION

×COUNT NUMBER OF READS IN YES AND NO PORTIONS OF PREMISE LINES
×COUNT NUMBER OF NO READS IN YES PORTIONS
×COUNT NUMBER OF YES READS IN NO PORTIONS
×COMPUTE CHI-SQUARE

	YES REGION	NO REGION
NUMBER EXPECTED	26	60
NUMBER OBSERVED	26	58
DEVIATION	0	2
CHI-SQUARE	0/26 +	4/60 = 0.07

PROBABILITY OF LARGER CHI-SQUARE (1 df) = 0.50

×PROVISIONAL DEFINITION OF CLONE DELINEATION ACCEPTED

VERIFICATION OF CLONE DEFINITIONS

×COUNT NUMBER OF READS IN VERIFY REGIONS OF PUNNET SQUARE
×COUNT NUMBER OF NO READS IN YES REGIONS
×COUNT NUMBER OF YES READS IN NO REGIONS
×COMPUTE CHI-SQUARE

	YES REGION	NO REGION
NUMBER EXPECTED	74	150
NUMBER OBSERVED	72	148
DEVIATION	2	8
CHI-SQUARE	4/72 +	64/150 = 0.48

PROBABILITY OF A LARGER CHI-SQUARE (1 df) = 0.50

×PROVISIONAL CLONAL DEFINITION IS VERIFIED

Figure 4—Explanation of statistical analysis of data obtained from plot FH-4.

The Challenges

A total of 5,543 challenges were made in the course of delineating the population of isolates into clones. Because the blind challenges contained 192 isolate × self challenges, the precision of the methodology could be investigated. The challenges were classified into two groups where compatibility (yes) is expected (isolate × self and isolates within clones) and two groups where noncompatibility (no) is expected (clones in plots and plots × plots). Because they were expected to be identical, "yes" was expected any time an isolate was selfed. "Yes" was also expected when two isolates shown by previous tests to belong to the same clone were challenged (verifying region of the Punnett square). "No" was expected if two clones separated by more than 500 meters were challenged according to current ideas about clone extent in western forests (Anderson and others 1979). "No" was expected if two previously defined clones from the same plot were challenged. Two kinds of disagreement were investigated: the readers recorded independently and could disagree with each other, or they could agree (in the read) on the result of the challenge but disagree with the expected result. The selfed isolates provide a control or check on the challenge methodology because the only presumed source of variation is the challenge system. The readers disagreed 4 percent of the time when a "yes" was expected from selfed challenges. The population of reads obtained from the clone-verification zones of the Punnett squares was virtually the same as reading selfed isolates (table 1).

When "yes" was expected from blind reading of selfed isolates, the readers agreed to disagree with the expectation 5 percent of the time. The clone-verification population was not significantly different from the selfed isolates (table 2), even though the former population showed reduced disagreement.

Clones will fail to grow together because they belong to different intersterility groups or they are different genotypes within the same group. Thus the level of reader differences with a "no" expectation was assessed. Disagreement among readers was significantly different between clones within plots and clones between plots (table 3), yet the difference was not significant when readers agreed to disagree with expected results (table 4).

Geographic Extent of Clones

The geographic extent of clones within a National Forest was examined by constructing challenges of coded isolates, in all possible combinations of isolates, obtained from the 12 National Forests. The challenges between clones from different plots resulted in 95 percent "no," 2 percent "yes," and 3 percent "maybe" (table 5). The National Forests varied from 0 percent to 8 percent double "yes" when it was expected that both would be "no."

Table 1—Disagreement between two readers of challenges between field isolates of *Armillaria* when isolates were expected to be compatible

Isolate populations	Number of challenges			Reader disagreement
	Yes and no	Two yes	Total	
				Percent
Selfed	7	176	183	3.8
Clone verification	17	427	444	3.8

$\chi^2 = 0.05$ Probability of larger χ^2 (1 df) = 0.90

Table 2—Accord in dissent with theoretical expectation between two readers of challenges between field isolates of *Armillaria* when isolates were expected to be compatible

Isolate populations	Number of challenges			Reader dissent
	Two no	Two yes	Total	
				Percent
Selfed	9	176	185	4.7
Clone verification	16	427	443	3.6

$\chi^2 = 0.26$ Probability of larger χ^2 (1 df) = 0.70

Table 3—Disagreement between two readers of challenges between field isolates of *Armillaria* when isolates were expected to be incompatible (challenges from verify zone-no region of clone-definition Punnett squares and clones between plots)

Isolate populations	Number of challenges			Reader disagreement
	Yes and no	Two no	Total	
				Percent
Verify zones	25	426	451	5.5
Clones between plots	102	3,106	3,308	3.1

$\chi^2 = 4.00$ Probability of larger χ^2 (1 df) = 0.05

Table 4—Accord in dissent with theoretical expectation between two readers of challenges between field isolates of *Armillaria* when isolates were expected to be incompatible (challenges from verify zone-no region of clone-definition Punnett squares and clones between plots)

Isolate populations	Number of challenges			Reader dissent
	Two yes	Two no	Total	
				Percent
Verify zones	17	426	443	3.8
Clones between plots	95	3,128	3,223	2.9

$\chi^2 = 0.76$ Probability of larger χ^2 (1 df) = 0.40

Table 5—Percentage of disagreement between two readers and agreement to disagree with expectation when *Armillaria* isolates from clones from different plots within a National Forest are challenged

National Forest	Number of challenges	No	Yes	Yes and no
		- - - - - - - *Percent* - - - - - - -		
Coeur d' Alene	153	96	2	2
Colville	256	100	0	0
Clearwater	76	98	1	1
Flathead	264	93	3	4
Kaniksu	154	93	6	1
Kootenai	232	89	8	3
Lolo	240	94	6	0
Nez Perce	262	92	2	6
Payette	395	95	2	3
St. Joe	554	96	1	3
Umatilla	568	96	1	3
Wallowa-Whitman	129	93	7	0
Total	3,283	95	2	3

Table 6—Characteristics of *Armillaria* clones obtained from Northern Rocky Mountain National Forests

Clone source	Number of clones	Percent of clones	Isolates per clone
Hardwood shrubs	42	24	1.2
Healthy and detritus conifer	64	36	1.4
Shrubs and conifers	32	18	4.1
Free soil rhizomorph	4	2	1.0
Pathogenic on conifers	22	12	1.4
Shrubs and pathogenic on conifers	13	7	4.3
Total clones	177		

Number of Clones per Plot

The average number of clones per plot was 2.3. The average number of isolates per plot was 5.4. Plot SJ-1 (table 16, appendix) provided 12 isolates and three clones. A nearby plot, SJ-4 about 17 km northwest, gave the largest number of clones, which was six among eight isolates.

Clone Habits

The clones were classified according to their host affiliations (table 6). Six mutually exclusive classes were established. The first included clones existing as rhizomorphs epiphytic on apparently healthy hardwood brush. The second was epiphytic on healthy conifers, as well as conifer detritus. The third class included epiphytic rhizomorphs on both conifers and hardwoods. The fourth was composed of clones from rhizomorphs unattached to any organic material in the soil. The fifth included clones that were restricted to conifers and connected to conifer pathogenicity by having both fan isolates and epiphytic growth on conifers that were showing symptoms from the *Armillaria* infection. A sixth class contained clones connected, as above, to conifer pathogenicity but were also found in epiphytic associations with hardwoods.

There are several trends associated with clone habit. The highest percentage of clones was found on healthy hardwoods and on healthy conifers and/or conifer detritus. These tended to be represented as single isolates (table 6). Clones sampled as multiple isolates tended to be on a variety of host types (shrubs and conifers in table 6). Likewise, pathogenic clones that occurred on both shrubs and conifers tended to exist on a variety of substrates, but those present only as conifer pathogens tended to occur as single isolates.

DISCUSSION

Clone Definitions

The vegetative samples used in this study of *Armillaria* spp., as they exist in the Northern Rocky Mountains, separated into 177 separate and distinct clones. We have yet to directly determine their taxonomic affiliations and some other important traits. As a research resource they provide a sizable population of cultures with demonstrated genetic interrelationships that will provide a population on which to build a classification system based on traits as expressed in culture. This should be done on a large population before species relationships are known. As such, it would allow fair and unbiased determination of the stability of the traits. After a classification based on stable traits is developed, it can be verified by determination of the species of each clone.

Vegetative collections of *Armillaria* spp. are believed to be diploid, but the occurrence of haploids has not been studied. These clones will provide an ideal resource on which to study this possibility—for example, haploids can be distinguished by their fluffy aerial mycelium (Anderson and Ullrich 1979; Korhonen 1978), haploid nuclei can be separated from diploid nuclei by microspectrophotometric techniques and nuclear volume measurements (Motta 1985; Peabody and Peabody 1984), and finally, a vegetative isolate that is haploid would behave differently than a diploid in challenge and mating tests.

The Challenge System

Results reported in this paper demonstrate that the reading of isolate compatibility is not without ambiguity; however, our level of error is consistent with that reported by others. Within our population of isolates it appears that about 5 percent of the interpretations between closely related genotypes (tables 1 and 2) are subject to error. This level is less than the 10 percent error level reported for similar interactions within closely related genotypes of *A. luteobubalina* (Kile 1983). Matings between supposedly compatible haploids also yield 5 to 10 percent ambiguous interpretations (Kile 1983; Korhonen 1978). Placing numerous unrelated cultures in the same confined space apparently did not lead to unstable challenge results. The system of challenges for field diploid isolates, as used in this study, will give repeatable results, and it provides a saving in time and resources compared to a single challenge per plate. The precision of our results reinforces the need to base clone delineation on repeated challenges of coded isolates.

The results of the expect-"no" analysis for both conditions (tables 3 and 4) can also be interpreted on the basis of ambiguous-separation between closely related genotypes. Both kinds of error indicate uncertain reads, and both kinds of errors are higher within plots than between plots. The interpretation of this pattern, however, is different than for the expect-"yes" case. An additional possible source of error in the expect-"no" case is the existence, in the field, of haploid vegetative isolates that could function as "testers." For example, where we use four isolates placed 5 mm apart within the same petri dish they could grow freely with any diploid of the same species and mating could occur, after which the haploid would not react with a different adjacent diploid clone. The existence of such haploids in a population believed to be diploid would generate unstable results, especially within the multiple isolate challenge system used by us. Some of these problems could be circumvented if mating reactions and mycelial growth pattern were also recorded; haploids produce a distinctive growth of aerial mycelium (Korhonen 1978; Ullrich and Anderson 1978).

As stated above, the verification system we present is predicated on the testing of diploid isolates. The existence of a haploid isolate would lead to inconclusive results. If two clones of the same species were being delineated, and one clone contained a haploid isolate, that haploid could grow with both clones. An unexpected "yes" reading would result. The same problem exists in testing the wider extent of clones. According to current theory, a haploid isolate could conceivably give a "yes" read against any diploid isolate of the same species from anywhere in the world. If one were expecting such a mating response, this situation could be monitored. At the very least, any future clonal delineation should include provisions for assessing mating and the presence of haploids in the test population. The existence of haploid isolates could explain the unusually high number of ambiguous readings between plot-to-plot clones within a National Forest (table 5). These challenges were made on an isolate-specific level before the clones were defined to provide data unbiased by reader knowledge. This system led to a random selection of isolate pairing. If a haploid was selected to represent the clone and it was paired against the same species from a distant plot, a "yes" (when a "no" was expected) would result. Even though most authors believe that vegetative field isolates are diploid (Kile 1983; Korhonen 1978; Ullrich and Anderson 1978; Wargo and Shaw 1985), one must keep in mind that most of these workers have made almost exclusive use of cultures derived from sporophores or from vegetative samples obtained in the vicinity of sporophores on sites where *Armillaria* spp. are in a pathogenic mode. In addition, it is theoretically possible that haploids could have arisen from diploids unstable in culture. This, however, does not appear to be the case, since the diploids of the genus have been shown to possess a high degree of genetic stability (Anderson and Ullrich 1982). Existence of these haploids may not be so surprising in light of the recent reports of haploid tissues in *Armillaria* spp. basidiocarps (Peabody and Peabody 1986). Our tentative conclusion based on the challenge behavior of some isolates is that our random samples of vegetative structures most probably included some haploids. A thorough understanding of the confirmed existence, distribution, behavior, and role of such haploid components would explain much about the genetics and epidemiology of the genus *Armillaria*.

Number of Clones per Plot

A most interesting finding is that an average of 2.3 clones per 0.04-ha plot was found. This result supports the current ideas regarding the distribution of clones and species. As seen in tables 7 thru 18 in the appendix, most plots contained one or two clones regardless of the number of isolates obtained. This argues for the coexistence of two species on most sites, since others have not found two clones of the same species to commonly coexist (Kile 1983; Korhonen 1978; Ullrich and Anderson 1978). Several sites had three clones and could represent the coexistence of three species. The final answer to this generalization will come with the confirmation of species affiliations of the sampled clones.

Clone Habits

Associations of clones by hosts and rhizomorph production (table 6) suggest four *Armillaria* spp. in our population of randomly collected isolates. One group of clones comprised 24 percent of all clones and was restricted to hardwood shrubs. These were generally found as a single rhizomorphic isolate. If the average number of rhizomorphic-derived cultures is a measure of tendency of a clone to produce rhizomorphs, then this group may signify a species that rarely produces rhizomorphs and is restricted to hardwood hosts. A second group was restricted to conifers and produced few rhizomorphs. It averaged 1.4 isolates per clone. This group was about evenly split on healthy conifers, conifer detritus, and in pathogenic mode. It included 48 percent of all clones. This pattern fits *A. ostoyae* as described by Rishbeth (1982) in England and Morrison and others (1985) in British Columbia. The third group produced an average of 4.1 isolates per clone. It was found on shrub detritus, conifer detritus, healthy shrubs, and healthy conifers. This group contained 18 percent of all clones and behaves like *A. bulbosa* in England (Rishbeth 1982) where it was found on both hardwoods and conifers. This species is a prolific producer of rhizomorphs, and is not pathogenic. This species was found in British Columbia but was restricted to hardwoods as shown by a sporophore population (Morrison and others 1985). The fourth group of clones was a prolific producer of rhizomorphs (4.3 isolates per clone) and occurred on shrubs, conifer detritus, healthy conifers, and in a pathogenic mode. Only 7 percent of the clones belonged to this group. This group may represent a species that produces many rhizomorphs, is pathogenic on conifers, and commonly exists in a saprophytic mode.

CONCLUSIONS

A population of clones belonging to the genus *Armillaria* has been defined. All isolates comprising the clones are secured in contaminant-free archival collections. An effective technique of challenging large numbers of isolates to delineate clones has been demonstrated and verified. Grouping of clones, based on the behavior of certain species, suggests the Northern Rockies is home for at least four species of the genus. Many avenues appear to be open for the confirmation of species affiliation in populations of vegetative isolates. Complete understanding of the life cycle, genetics, and ecological role of the *Armillaria* complex will come with a thorough investigation of vegetative populations obtained from healthy as well as diseased stands.

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APPENDIX: DELINEATION INTO CLONES OF *ARMILLARIA* SPP. ISOLATES COLLECTED ON 12 NATIONAL FORESTS

Table 7—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Coeur d' Alene National Forest in Idaho

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
CD-1	8	7	3	18	16	22	20	0.40	12	12	20	20	0.00
CD-2	7	5	2	10	10	2	2	.00	8	8	12	12	.00
CD-3	7	3	2	6	6	4	4	.00	0	0	8	8	FIT[1]
CD-4	8	5	2	14	14	2	2	.00	22	20	12	11	.27
CD-5	9	1	1	—	—	—	—	—	—	—	—	—	—
CD-6	2	0	—	—	—	—	—	—	—	—	—	—	—
CD-7	1	0	—	—	—	—	—	—	—	—	—	—	—
CD-7a	4	4	2	8	8	8	8	.00	4	4	6	6	.00
CD-8	7	0	—	—	—	—	—	—	—	—	—	—	—
Plot $\bar{X}$	5.9	2.8	2.0										

[1] Close or exact fit not testable by chi square.

Table 8—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Colville National Forest in Washington

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
CO-1H	6	5	3	14	14	16	16	0.00	0	0	10	10	FIT[1]
CO-1	6	6	3	12	12	30	26	1.20	10	10	28	28	0.00
CO-2	1	1	1	—	—	—	—	—	—	—	—	—	—
CO-3	7	6	1	32	32	0	0	FIT	44	44	0	0	FIT
CO-4	4	4	3	2	2	14	14	.00	0	0	8	8	FIT
CO-5	4	4	2	4	4	8	6	.50	4	2	4	4	1.00
CO-6	0	—	—	—	—	—	—	—	—	—	—	—	—
CO-7	9	8	1	30	30	0	0	FIT	74	74	0	0	FIT
CO-8	10	9	3	36	25	26	21	4.32	54	52	36	29	1.44
Plot $\bar{X}$	5.9	5.4	2.1										

[1] Exact fit not testable by chi square.

Table 9—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Clearwater National Forest in Idaho

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
CW-1	7	7	1	22	22	0	0	FIT[1]	50	50	0	0	FIT
CW-2	6	0	—	—	—	—	—	—	—	—	—	—	—
CW-3	6	0	—	—	—	—	—	—	—	—	—	—	—
CW-4	6	0	—	—	—	—	—	—	—	—	—	—	—
CW-5	7	5	2	8	8	4	4	0.00	8	8	12	12	0.00
CW-6	2	2	1	4	4	0	0	FIT	—	—	—	—	—
CW-7	7	3	3	0	0	10	9	FIT	—	—	—	—	—
CW-8	7	1	1	—	—	—	—	—	—	—	—	—	—
Plot $\bar{X}$	6.0	2.3	1.6										

[1] Close or exact fit not testable by chi square.

Table 10—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Flathead National Forest in Montana

National Forest	Number samples	Number isolates	Number clones	Provisional					Verification				
				Yes		No		Chi square	Yes		No		Chi square
				E	O	E	O		E	O	E	O	
FH-1	0	—	—	—	—	—	—	—	—	—	—	—	—
FH-2	0	—	—	—	—	—	—	—	—	—	—	—	—
FH-3	5	1	1	—	—	—	—	—	—	—	—	—	—
FH-4	12	11	4	26	26	60	58	0.07	74	72	150	142	0.48
FH-5	10	6	3	28	28	14	14	.00	18	18	58	58	.00
FH-6	9	7	3	52	40	32	19	8.05	52	33	72	51	13.07
FH-7	6	5	5	0	0	74	60	FIT[1]	—	—	—	—	—
FH-8	2	0	—	—	—	—	—	—	—	—	—	—	—
Plot $\bar{X}$	7.3	5.0	3.2										

[1]Close or exact fit not testable by chi square.

Table 11—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Kaniksu National Forest in Idaho

National Forest	Number samples	Number isolates	Number clones	Provisional					Verification				
				Yes		No		Chi square	Yes		No		Chi square
				E	O	E	O		E	O	E	O	
KN-1	3	3	1	6	6	0	0	FIT[1]	2	2	0	0	FIT
KN-1A	2	2	2	0	0	4	4	FIT	—	—	—	—	—
KN-2	2	2	1	10	10	0	0	FIT	—	—	—	—	—
KN-3	7	7	3	12	8	16	16	1.33	22	22	26	26	0.00
KN-4	10	10	3	10	10	24	20	.67	30	30	46	37	1.76
KN-5	7	7	2	14	14	4	3	.25	26	26	22	22	.00
KN-6	0	—	—	—	—	—	—	—	—	—	—	—	—
KN-7	1	0	—	—	—	—	—	—	—	—	—	—	—
KN-8	6	6	2	16	16	6	4	.67	18	16	16	13	.78
Plot $\bar{X}$	4.8	4.6	2.0										

[1]Exact fit not testable by chi square.

Table 12—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Kootenai National Forest in Montana

National Forest	Number samples	Number isolates	Number clones	Provisional					Verification				
				Yes		No		Chi square	Yes		No		Chi square
				E	O	E	O		E	O	E	O	
KT-1	5	4	1	12	12	0	0	FIT[1]	8	8	0	0	FIT
KT-2	6	5	2	8	8	2	2	0.00	10	10	26	22	0.61
KT-3	8	8	5	12	12	68	60	.94	2	2	48	46	.80
KT-4	5	5	1	12	11	0	0	FIT	24	23	0	0	FIT
KT-5	3	2	1	6	6	0	0	FIT	—	—	—	—	—
KT-6	0	0	0	—	—	—	—	—	—	—	—	—	—
KT-6A	2	2	1	4	4	0	0	FIT	—	—	—	—	—
KT-7	6	5	3	6	6	26	18	2.46	2	2	18	13	1.39
KT-8	0	—	—	—	—	—	—	—	—	—	—	—	—
KT-8A	—	—	—	—	—	—	—	—	—	—	—	—	—
Plot $\bar{X}$	3.5	4.4	2.0										

[1]Close or exact fit not testable by chi square.

Table 13—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Lolo National Forest in Montana

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	**Number samples**	**Number isolates**	**Number clones**	**E**	**O**	**E**	**O**	**Chi square**	**E**	**O**	**E**	**O**	**Chi square**
LO-1	3	0	—	—	—	—	—	—	—	—	—	—	—
LO-2	—	—	—	—	—	—	—	—	—	—	—	—	—
LO-2A	9	9	2	18	18	4	4	0.00	18	18	14	7	2.33
LO-3	—	—	—	—	—	—	—	—	—	—	—	—	—
LO-3A	13	7	1	18	16	0	0	FIT[1]	24	24	0	0	FIT
LO-4	7	4	2	4	4	8	8	.00	0	0	4	2	FIT
LO-5	7	6	3	6	6	8	8	.00	8	6	24	22	.67
LO-6	3	3	3	0	0	12	12	FIT	—	—	—	—	—
LO-7	7	4	2	6	6	4	4	.00	2	2	2	2	.00
LO-8	7	7	3	12	12	8	8	.00	28	24	18	16	.79
Plot $\bar{X}$	5.6	5.0	2.3										

[1] Close or exact fit not testable by chi square.

Table 14—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Nez Perce National Forest in Idaho

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	**Number samples**	**Number isolates**	**Number clones**	**E**	**O**	**E**	**O**	**Chi square**	**E**	**O**	**E**	**O**	**Chi square**
NP-1	7	7	1	16	16	0	0	FIT[1]	24	24	0	0	FIT
NP-2	4	2	2	0	0	6	6	FIT	—	—	—	—	
NP-3	6	4	2	6	6	6	6	0.00	2	2	4	4	0.00
NP-4	7	6	1	12	12	0	0	FIT	28	28	0	0	FIT
NP-5	6	6	2	14	12	8	8	.29	10	10	26	26	.00
NP-6	9	6	4	8	8	8	8	.00	2	2	18	14	.89
NP-7	5	3	1	12	12	0	0	FIT	—	—	—	—	—
NP-8	7	3	2	6	6	2	2	.00	0	0	6	6	FIT
Plot $\bar{X}$	6.4	4.6	1.9										

[1] Exact fit not testable by chi square.

Table 15—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Payette National Forest in Idaho

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	**Number samples**	**Number isolates**	**Number clones**	**E**	**O**	**E**	**O**	**Chi square**	**E**	**O**	**E**	**O**	**Chi square**
PA-1	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-1-7	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-1B	8	4	2	4	4	6	6	0.00	0	0	8	8	FIT[1]
PA-2	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-3	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-4	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-5	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-6	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-7	0	—	—	—	—	—	—	—	—	—	—	—	—
PA-7A	8	5	2	10	10	4	4	.00	8	8	10	10	.00
PA-8	4	0	—	—	—	—	—	—	—	—	—	—	—
PA-8A	13	8	3	12	12	12	12	.00	24	24	30	30	.00
Plot $\bar{X}$	3.0	4.25	2.3										

[1] Exact fit not testable by chi square.

Table 16—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the St. Joe National Forest in Idaho

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
SJ-1	14	12	3	14	14	10	10	0.00	62	51	78	71	2.59
SJ-2	9	4	1	26	23	0	0	FIT[1]	16	12	0	0	FIT
SJ-2A	3	3	1	10	10	0	0	FIT	4	4	0	0	FIT
SJ-3	9	6	3	6	6	18	18	.00	2	2	14	10	1.14
SJ-4	9	8	6	14	12	70	68	.34	0	0	66	57	FIT
SJ-5	7	0	—	—	—	—	—	—	—	—	—	—	—
SJ-6	0	—	—	—	—	—	—	—	—	—	—	—	—
SJ-7	7	4	2	12	12	6	6	.00	2	2	10	10	.00
SJ-8	8	7	4	10	10	58	58	.00	12	10	14	14	1.33
Plot $\bar{X}$	7.3	5.5	2.9										

[1] Close or exact fit not testable by chi square.

Table 17—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Umatilla National Forest in Oregon and Washington

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
UM-1	2	2	2	0	0	8	6	FIT[1]	—	—	—	—	
UM-1A	4	4	2	4	4	2	2	0.00	4	4	4	4	0.00
UM-2	4	1	—	—	—	—	—	—	—	—	—	—	—
UM-3	0	—	—	—	—	—	—	—	—	—	—	—	—
UM-4	6	6	3	18	18	8	8	.00	10	10	10	8	.40
UM-5	4	3	1	18	18	0	0	FIT	6	5	0	0	FIT
UM-6	6	5	3	12	11	10	10	.08	0	0	12	12	FIT
UM-7	7	6	4	14	14	32	30	.13	8	8	26	25	.04
UM-8	10	10	2	20	18	4	4	.20	26	23	26	26	.35
Plot $\bar{X}$	4.8	4.6	2.4										

[1] Close or exact fit not testable by chi square.

Table 18—Delineation into clones of vegetative samples of *Armillaria* spp. collected on the Wallowa-Whitman National Forest in Oregon

				Provisional					Verification				
				Yes		No			Yes		No		
National Forest	Number samples	Number isolates	Number clones	E	O	E	O	Chi square	E	O	E	O	Chi square
WW-1	0	—	—	—	—	—	—	—	—	—	—	—	—
WW-2	5	4	1	12	12	0	0	FIT[1]	6	6	0	0	FIT
WW-3	5	5	2	6	6	6	6	0.00	4	4	10	10	0.00
WW-4	8	6	2	12	12	6	6	.00	34	30	10	10	.48
WW-5	9	8	4	14	14	28	28	.00	2	2	28	24	.57
WW-6	0	—	—	—	—	—	—	—	—	—	—	—	—
WW-7	0	—	—	—	—	—	—	—	—	—	—	—	—
WW-8	0	—	—	—	—	—	—	—	—	—	—	—	—
Plot $\bar{X}$	3.4	5.8	2.3										

[1] Exact fit not testable by chi square.

McDonald, Geral I.; Martin, Neil E. 1988. *Armillaria* in the Northern Rockies: delineation of isolates into clones. Res. Pap. INT-385. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Research Station. 13 p.

Isolates derived from *Armillaria* spp. rhizomorphs and fans collected within a 300-mile radius of Moscow, ID, were delineated into clones. The 394 isolates taken from 87 randomly located 0.04-ha plots were assigned to 177 clones. Multiple blind assessments of isolates among clones produced an overall reading error of about 5 percent. An average of 5.4 isolates per plot yielded two to three clones per plot, leading to the conclusion that most plots supported two or three species of *Armillaria*. The clones seemed to fall into four groups with regard to host affinities, pathogenicity, and rhizomorph production. This suggests that the Northern Rocky Mountains are home to four *Armillaria* spp.

KEYWORDS: isolate compatibility, *Armillaria* specification, statistical analysis of clone definitions

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